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NEW APPROACH FOR PAPAYA LATEX STORAGE WITHOUT VIRUS DEGRADATION

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ABSTRACT

Papaya meleira virus (PMeV) is the causal agent of papaya (*Carica papaya* L.) sticky disease, which has been detected through analysis of its double-stranded RNA (dsRNA) genome from plant latex. In this work we demonstrate that PMeV dsRNA is protected during 25 days when latex is diluted in citrate buffer pH 5.0 (1:1 v/v) and maintained at -20ºC. At the same temperature, some protection was observed for pure latex or latex diluted in ultra-pure water. Conversely, the dsRNA was almost completely degraded after 25 days when maintained at 25ºC, indicating the need for freezing. The proper procedures to collect and store papaya latex described here will contribute to efficient and large scale use of molecular diagnosis of PMeV.

Keywords: papaya sticky disease, papaya latex, *Papaya meleira virus*.

Sticky disease or "meleira" is an important phytosanitary problem in papaya (*Carica papaya* L.) production and the economical consequences are drastic for several Brazilian States. The disease etiology arises from infection by *Papaya meleira virus* (PMeV) which has a 12 kbp double-stranded RNA (dsRNA) genome (4,10). Using electron microscopy, PMeV was demonstrated colonizing only diseased papaya laticifers and its dsRNA could be extracted from infected latex (4,10). Papaya plants with this disease show spontaneous exudation of a fluid and translucent latex from fruits and leaves and the first notable symptom of the disease is the occurrence of small necrotic lesions on young leaf edges caused by latex oxidation after atmospheric exposure (9).

Visual observation of meleira symptoms is often used as a disease management strategy for cultivated areas followed by the eradication of symptomatic papaya plants (roguing) (8,9). When roguing is properly undertaken the sticky disease is considerably controlled. Nevertheless, an inherent infection feature compromises the roguing efficiency as the symptoms are only displayed after papaya flowering. Infected but asymptomatic plants can, therefore, remain in the field as a virus source to infect nearby healthy plants. In fact, even in orchards where roguing is well performed, about 20% of the plants may be infected (9).

Aiming at earlier identification of infected plants, two different molecular approaches have been developed. The first strategy is based on the extraction of PMeV genomic dsRNA from the plant latex using organic solvents followed by gel electrophoresis (6). Although such a method does not use nucleic acid amplification, it has an attractive sensitivity considering the virus occurrence in high concentration in the latex (4,6,7,10). We have observed an intense 12 kbp nucleic acid band in agarose gel through the dsRNA extraction from papaya latex about 15 days after PMeV inoculation (7). This method has been used both to analyze asymptomatic plants and to confirm field diagnosis. Recently, primers specific for PMeV were designed based on nucleotide sequences of the viral dsRNA. Through RT-PCR, a 669-nucleotide fragment was amplified and showed a high similarity with other viral RNA-dependent RNA polymerases after sequencing (1).

Efficiency of either molecular diagnosis strategy, direct PMeV dsRNA extraction or amplification by RT-PCR, depends on previous latex processing. Usually, papaya producers send fruits, leaves and stems inside plastic bags to diagnostic laboratories representing an expensive and time-consuming procedure. Sometimes, the stress during plant transport inhibits latex exudation and makes diagnosis impracticable. Once in the

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laboratory, the latex is collected into a microtube containing acidic buffer followed by immediate processing and analysis by gel electrophoresis (6). As the effect of latex biochemistry on the dsRNA is not known, all procedures are quickly performed. Latex can be considered a great redox environment and its exudation is accompanied by direct activation of several enzymes. Besides enzymatic activity, other latex compounds may also damage PMeV particles (5). Therefore, latex must be correctly processed to avoid dsRNA destruction and consequent diagnosis failure. For that reason, in this study we have evaluated different procedures for latex storage prior to PMeV molecular diagnosis. The effect of solvents and temperature on PMeV dsRNA stability was compared and the results are discussed.

Papaya plants were grown under controlled conditions at the Incaper Experimental Field in Northern Espírito Santo State. Using a new commercial razor blade for each fruit, healthy and diseased fruits were superficially incised and the latex collected into separate microtubes for each sample. Samples were undiluted or diluted in citrate buffer pH 5.0 (1:1 v/v) or ultra-pure water (1:1 v/v) and stored at -20ºC (freezer), 5ºC (refrigerator) or 25ºC (room temperature). Molecular analysis to reveal the dsRNA from PMeV was performed as previously described (6) every 5 days, for a total period of 25 days. For each individual analysis, 50 µl of undiluted or 100 µl diluted latex it was used.

Samples of pure latex, latex diluted in citrate buffer pH 5.0 or latex diluted in ultra-pure water showed a distinct nucleic acid band of about 12 kbp equivalent to PMeV dsRNA, as seen in other works (4,6,7,9), and no band was observed in samples from healthy plants with no diseased symptoms (Fig. 1).

Band intensity in the electrophoresis gel showed a progressive decrease for samples stored at 25ºC. After 25 days the band was almost undetectable as a result of dsRNA degradation (Fig. 1). Therefore, latex at 25ºC drastically damaged the PMeV dsRNA. This result contrasts with the known stability of dsRNA molecules, including stability at different temperatures. For example, it has been shown that PMeV dsRNA specifically melts at 96ºC (10). However, the degradation observed in this work may be a combined result of temperature with latex compounds, such as enzymes, phenols, isoprens and alkaloids (2). Actually, this experiment was also performed to verify the influence of temperature on the papaya latex. We had observed that infected latex became dark brown under high temperatures (50-70ºC for 15 minutes) while healthy latex remained white (data not shown).

Latex at low temperature (-20ºC and 5ºC) had the dsRNA preserved throughout the entire experiment as identical bands were observed for the different analyzed periods (Fig. 1). It is notable that samples diluted in citrate buffer pH 5.0 provided more intense nucleic acid bands than those observed for pure latex or latex diluted in ultra-pure water (Fig. 1). Such a result

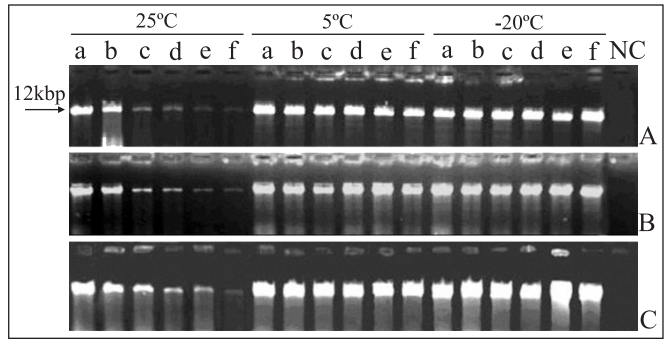

Figure 1. Nucleic acids extracted from papaya sticky diseased latex submitted to different dilution and storage conditions. (A) Pure latex, (B) latex diluted in ultra-pure water or (C) diluted in citrate buffer pH 5.0 were storaged at different temperatures, indicated at figure top, and analyzed after 1 (a), 5 (b), 10 (c), 15 (d), 20 (e) and 25 (f) days. Latex from healthy plants was used as negative control (NC).

may be related to increased RNA stability in acidic buffers (3). Also, acidification may inhibit latex enzymes resulting in dsRNA protection. Comparing latex diluted in citrate buffer pH 5.0 followed by storage at 25, 5 or -20ºC, it was observed that this last condition provided the best result for identification of the nucleic acid band in the gel (Fig. 1), suggesting the greatest dsRNA integrity. Therefore, this useful low pH effect is increased by low temperature.

In conclusion, latex can be collected in the field by papaya producers through incisions using a razor blade on leaf stalks, stems (mainly from the apex) or fruits. Better molecular diagnosis is achieved when latex is diluted in citrate buffer pH 5.0 (1:1 v/v) and maintained at -20ºC. Under such condition, this latex can be stored for about 25 days without dsRNA degradation. Latex should be diluted with ultra-pure water or left pure only if the citrate buffer is unavailable. Nevertheless, this is a factor to be considered for PMeV dsRNA detection from papaya whose virus load is still low, for example at early infection stages.

This communication is the first to describe conditions to drastically reduce sample volumes to be sent to diagnostic laboratories, making "meleira" diagnosis more accessible to papaya producers. Also, proper latex dilution and storage conditions assure more accurate diagnosis.

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RESUMO

Nova metodologia de armazenamento do latex de mamão sem degradação viral

Papaya meleira virus (PMeV) é o agente etiológico da meleira do mamoeiro (*Carica papaya* L.), cujo diagnóstico é feito através da detecção do RNA dupla-fita (dsRNA) viral a partir do látex das plantas. Neste trabalho é demonstrado que o dsRNA do PMeV é protegido durante 25 dias quando diluído em tampão citrato pH 5.0 (1:1 v/v) seguido de armazenamento à -20ºC. Nesta mesma temperatura, o dsRNA foi parcialmente protegido quando o látex foi diluído em água ultra-pura ou mantido puro. Ao contrário, quando as amostras foram mantidas à 25ºC, observou-se uma degradação progressiva do dsRNA, com ausência de bandas após 25 dias, indicando a necessidade do congelamento do látex. Os procedimentos de coleta e armazenamento do látex descritos neste trabalho contribuem para a eficiência e uso em larga escala do diagnóstico molecular do PMeV.

Palavras chave: meleira do mamoeiro, látex do mamão, *Papaya meleira virus*.

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